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APR 27 2001
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## REMARKS

Claims 1-49 are pending. Reconsideration and allowance are requested.

The amendment to claim 15 corrects a typographical error and, thus, no new matter has been added.

Prosecution in this application was reopened by the mailing of Paper No. 22. Because it was believed that a personal interview between the Examiner and the inventor would aid in clarifying what was taught in this specification, the undersigned and Prof. Dymecki met with Examiner Baker on March 29, 2001. The Examiner's courtesy in granting the interview and explaining her enablement rejection are gratefully acknowledged. As noted in the Interview Summary (Paper No. 23), Applicant's arguments and evidence are being submitted now for formal consideration by the Examiner.

On page 2 of the Office Action, claims 1-49 were rejected under Section 112, first paragraph, as allegedly "containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." Applicant traverses.

The Examiner contends that this specification does not teach how to produce "functional recombination" in transgenic mice. It appears that the Examiner assumes "functional recombination" requires activation of a functional gene. But it would be clear to a person skilled in the art that Flp-mediated recombination resulting in gene activation (i.e., a "functional" gene) is only one objective of the invention. It is not essential to the invention's operability because cell marking and lineage tracing can be accomplished by recombination of an integrated DNA substrate and, for example, either direct detection of the recombined DNA substrate or deletion of a histochemical marker. See description of generation of *Wnt1::FLP* transgenic mice on page 45 of the specification and the results reported by Applicant (Dymecki, Develop. Biol., 201:57-65, 1998; Rodriguez and Dymecki, Neuron 27:475-486, 2000). The credibility of such a utility is admitted by the Examiner on page 2 of the Action.

In Example 2, Applicant showed that (a) recombination was only detected in mice containing recombinase and the Flp-recognition sequences and (b) recombination was site specific and precise. See pages 39-40 of the specification. Recombination could be detected despite the poor expression of h*ACTB* regulatory sequences

driving *lacZ* and the inability to stain cells with XGal. See page 45 of the specification.

In claim 4, the result of recombination is a transgenic mouse containing at least two diploid cells with different numbers of Flp-recombination sequences (i.e., chimeric or mosaic mice) is recited. Thus, an organ or tissue showing chimerism or mosaicism in the number of Flp-recombination sequences may be used to trace cell lineages without requiring activation of gene expression. See pages 4-5 of the specification ("A chimeric or mosaic transgenic non-human mammal may contain cells with different numbers of Flp-recognition sequences due to Flp-mediated recombination").

In claim 11, a drug selectable marker is excised by the Flp recombinase system. This allows a two-step selection scheme in producing transgenic mice by gene replacement using homologous recombination (see pages 21 and 43 of the specification discussing Fiering et al., 1995).

A "functional" recombination product as defined by the Examiner (i.e., activation of gene expression by recombination) is also not necessary to achieve the objectives of translocation between chromosomes, excision of a gene to create a null mutation, and insertion of a transgene. See pages 5-6 of the specification.

Therefore, production of a "functional" recombined gene product as defined by the Examiner is not required for operability of the invention because it is capable of other practical utilities. As discussed below, however, the invention can be used to activate expression of a transgene.

Arguments on pages 3-4 of the Action about the alleged unpredictability of achieving a "desired level of transgene expression in appropriate tissues" is not relevant to operation of the invention. Such objections would apply against all transgenic mice, not just those using the Flp recombinase system. In Applicant's Example 2, it was concluded on page 45 of the specification that "lack of βGal activity associated with the observed recombination most likely reflects a posi-tion effect on transgene transcription exerted by the genomic integration site since only one in four control *FRTZ-product* mouse lines expresses βGal." The recombined transgene was not sufficiently active to be used for cell marking by XGal stain when h*ACTB* regulatory sequences from the human β-actin gene was used to direct transgene expression. Thus, the objections raised in the Action do not render Applicant's invention use-

less. Instead, routine screening of additional lines of transgenic mice can identify a chromosomal integration site that supports a higher level of transgene expression. See page 45 of the specification.

It would also be known to a person skilled in the art that a histochemical reaction using βGal enzyme activity and XGal stain is not a sensitive assay for detecting recombination events and gene activation. In contrast, Applicant has used Southern blotting or PCR amplification with primers that detect the recombined target locus as sensitive assays to detect recombination events. See pages 39-41 of the specification. Similarly, RT-PCR would be able to detect a low level of transgene expression that might be undetectable by histochemical staining of cells.

Alternatively, activation of gene expression may be accomplished by choosing a transgene with greater biological activity (e.g., a more active Flp recombinase or other genes whose biological effects are easier to detect than βGal) or a regulatory sequence that is stronger than that of the human β-actin gene. For example, pages 15-17 of the specification describe the use of a variety of different regulatory regions and strategies to control expression of Flp recombinase or other transgenes. In particular, a regulatory region from a HMG-CoA reductase gene can be used as an alternative to the β-actin regulatory region used in Example 2.

Use of a Flp recombinase with enhanced thermostability (FLPe) and a regulatory sequence (*Hmgcr*) from the HMG-CoA reductase gene is demonstrated in Applicant's more recent publications (Rodriguez et al., Nature Genet. 25:139-140, 2000; Farley et al., Genesis 28:106-110, 2000). Such modifications are taught in the specification and are routinely made by a person skilled in the art. By the teachings of the specification, FLPe is a Flp transgene (see page 15) and the *Hmgcr* sequence is a regulatory sequence (see page 17). It is understood for purposes of claim construction and infringement analysis, however, that Applicant's claims are not limited to the Flp transgenes or regulatory regions that are exemplified in the specification.

Thus, the evidence of record shows that activation of transgene expression (i.e., "functional" recombination as defined by the Examiner) can be achieved by the invention. Even by the relatively insensitive histochemical assay using βGal enzyme and XGal stain, the transgenic mice of Example 2 can be modified in a manner taught in the specification and predicted by Applicant to show such gene activation.

Applicant's specification demonstrates that recombination mediated by Flp recombinase is functional because the reaction's product is site specific and precise with respect to the two Flp-recognition sites that have undergone recombination. In contrast to the statement on page 5 of the Action ("The problems encountered with respect to producing functional recombination suggest either imprecise recombination, wherein recombination does not result in gene activation, or inefficient recombination, wherein gene inactivation is only partial"), the more likely explanation for the lack of cell staining is an undetectable level of β-Gal activity.

Applicant stresses that the above results are being submitted only to rebut the Examiner's assertion that the invention is inoperable. These post-filing publications do not represent added statements to enable use of the invention because, as would be understood by the Examiner, such post-filing statements would not be effective when it is the specification as originally filed that must teach the person skilled in the art how to make and use the claimed invention. It is Applicant's specification that enables a person skilled in the art how to make and use the claimed invention.

There is also an assertion on page 5 of the Action that Claim 1 is not enabled because the specification does not teach how to use "a transgenic mouse having only an Flp recombinase transgene but not Flp-recognition sites." This is not correct because the specification teaches on page 20 that a genetic system with two lines of transgenic mammals, a first with a Flp transgene and a second with a transgene that contains at least one Flp-recognition site, may be used. Example 2 of the specification illustrates such a genetic system to produce mice with two transgenes.

The objection to Dr. Hammer's declaration on pages 5-6 of the Action is not relevant to operability of Applicant's invention because the experiments described therein were not successful. In the absence of Flp-mediated recombination in the transgenic mice produced by Dr. Hammer, no conclusions can be drawn about the transgenic mice described in this application.

Page 6 of the Action asserts that cell lineage marking is not a specific utility because "any piece of DNA inserted into the genome can be used to trace cell lineage." This assertion is also not correct because integration of a piece of DNA into the genome which is incapable of undergoing a change (e.g., Flp-mediated recombination) is not a marker for cell lineage. All cells of transgenic mice containing such "a piece of DNA" would be genetically identical. Therefore, there would be no genetic

distinction between two different cell lineages of a trangenic mouse. The invention has a specific, practical utility that "any piece of DNA inserted into the genome" does not perform.

Applicant respectfully requests withdrawal of the Section 112 rejection for the foregoing reasons.

Finally, Applicant respectfully repeats the request for review of the formal drawings submitted in this application. Return in the next Office Action of a Form PTO-948 completed by the Official draftsperson is respectfully requested.

Having fully responded to the Office Action (Paper No. 22), Applicant submits that the pending claims are allowable and an early Notice to that effect is earnestly solicited. The Examiner is invited to contact the undersigned if further information is needed.

Respectfully submitted,

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## APPENDIX

## MARKED-UP VERSION TO SHOW CHANGES

**IN THE CLAIMS:**

Claim 15 is amended as follows.

15. (2 x Amended) The transgenic mouse according to Claim 12, wherein said another transgene is [the purified nucleic acid further comprises a sequence] selected from the group consisting of genes controlling differentiation of a cell or development of an organism, genes required for viability of a cell or organism, cytokine genes, neurotransmitter or neurotransmitter receptor genes, oncogenes, tumor suppressor genes, selectable markers, and histochemical markers.